Table 1

Summary of source water and water treatment method for the drinking water supplies from 15 cities in Kansai, Japan.

Points	Source	Treatment method
1	Lake Biwa	Sodium hypochlorite, coagulation, sedimentation, sand filtration
2–3	Uji River	Ozone, coagulation, sedimentation, sand filtration, activated carbon, post-chlorination
4–6	Katsura River	Sodium hypochlorite, coagulation, sedimentation, sand filtration
7	Kizu River	Sodium hypochlorite, coagulation, sedimentation, sand filtration
8–9	Ground water	Sodium hypochlorite, coagulation, sedimentation, sand filtration
10–15	Yodo River	Ozone, coagulation, sedimentation, sand filtration, activated carbon, post-chlorination

2.2. Sampling

Tap water samples from Kyoto University were used for method development. Thirty tap water samples were collected from one city in Shiga Prefecture (Otsu; No. 1 in Fig. 1), six cities in Kyoto Prefecture (Uji, Joyo, Nagaokakyo, Muko, Otokuni and Yawata; Nos. 2–7 in Fig. 1) and eight cities in Osaka Prefecture (Ibaraki, Takatsuki, Settsu, Mishima, Moriguchi, Kadoma, Neyagawa and Hirakata; Nos. 8–15 in Fig. 1) in May 2013. The treatment processes used in the cities are presented in Table 1. Ozone and biological activated carbon were used in cities 2–3 and 10–15, while the NaOCl method was used in other cities (Table 1).

A 500 mL sample of tap water was collected from each location using a polypropylene disposable bottle that had been pre-rinsed with methanol and MQ water.

To avoid formation of additional HAAs and prevent HAA biodegradation, 100 mg L^{-1} of ammonium chloride was added immediately after sample collection to quench residual chlorine (Pepich et al., 2004). All the samples were stored at 4 °C and analyzed within 14 days.

2.3. Extraction and derivatization of HAAs in tap water samples

An aliquot (80 mL) of each water sample and 100 µL of 5 µg mL^{-1} 2,3-dibromopropionic acid were placed in a 100 mL separation funnel. The solution was saturated with 1.0 g of sodium chloride and acidified to pH 0.5 with 500 µL of hydrochloric acid. Next, the HAAs were extracted by vigorous shaking with 8 mL of MTBE for 3 min. The organic layer was collected in a distillation

flask and the aqueous layer was extracted again with 8 mL of MTBE for 3 min. The organic layers were combined and concentrated to 100 µL on a rotary evaporator. The residue was transferred into a screw cap vial, and 500 µL of the derivatization reagent (3% PFBBr) and 3 mg of potassium carbonate were added. The mixture was derivatized for 30 min at 60 °C in a forced convection oven. After heating, sample vials were cooled on aluminum cooler blocks at 4 °C immediately.

2.4. Instrumental analysis

After adding 10 µL of 5 ng $µL^{-1}$ 11H-eicosafluoroundecanoic acid as an internal standard, the derivatized samples were analyzed using gas chromatography–mass spectrometry (GC/MS) in selected ion monitoring mode (Agilent 6890GC/5973 MSD inert, Agilent Technologies Japan, Ltd., Tokyo, Japan). A 2 µL aliquot of the solution was injected into the GC/MS. Pentafluorobenzyl esters were separated on a HP-5MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Agilent Technologies Japan, Ltd.) with helium as the carrier gas (99.9999% purity; Air Liquide Japan Ltd., Tokyo, Japan). Splitless injections were performed with an inlet temperature of 240 °C, and the split vent was opened after 1 min. The oven temperature was initially held at 50 °C for 1.5 min, then increased to 150 °C at 20 °C min^{-1}, and then to 280 °C at 30 °C min^{-1}. NCI was used to ionize the HAA pentafluorobenzyl esters with methane (>99.9995% purity; Air Liquide Japan Ltd.) as the reagent gas (2 mL min^{-1}). The ion source temperature was maintained at 150 °C. The target ions for determination of the HAAs with NCI are summarized in Table 2.

2.5. Quality assurance, total extraction recovery, method detection limit and optimization of derivatization

MQ water was used as the procedural blank control, and was analyzed after every 10 samples (n = 3). The procedural blank was extracted using the process described in Section 2.3 and three replicate procedural blanks were prepared independently. The mean blank signal was subtracted from the calculated sample concentration only if the calculated sample concentration was three times higher than the method quantification limit (MQL).

The recoveries of the HAAs were examined by spiking 40 ng of each corresponding standard compound into an aliquot of MQ (80 mL), followed by extraction.

The instrumental detection limit (IDL, ng) is defined as the mass of the analyte producing a peak with a signal-to-noise ratio of

Table 2

Quality assurance for HAAs analyses in Milli-Q water.

Compound	Quantification ions(confirmation ions) m/z	Instrumental detection limit[a] (ng) (S/N = 3)	Mean recovery % and (SD)[b] of the analytes, n = 5	Procedural blank (SD) (ng L^{-1}), n = 3	MDL (ng L^{-1})[c]	MQL (ng L^{-1})[c]
TCAA	161 (163)	0.002	99 (12)	22 (6.1)	41	84
BDCAA	79 (81)	0.03	93 (11)	ND	94	250
DBCAA	79 (81)	0.03	89 (14)	ND	94	250
TBAA	79 (81)	0.03	91 (17)	ND	94	250
DBAA	217 (219)	0.001	95 (14)	26 (7.0)	47	96
DCAA	127 (129)	0.001	94 (12)	20 (6.1)	38	81
BCAA	173 (171)	0.001	98 (11)	6 (0.5)	8	11
MCAA	93 (95)	0.002	92 (13)	21 (7.4)	43	95
MBAA	137 (139)	0.001	97 (11)	16 (1.1)	20	27

SD: standard deviation; MDL: method detection limit; MQL: method quantification limit; ND: not detected.

[a] 2 µL injection; Instrumental detection limit (IDL) is defined as the mass of the analyte producing a peak with a signal-to-noise ratio of three.

[b] The recoveries of the HAAs were examined by spiking 40 ng of each corresponding standard compound into an aliquot of Milli-Q water (80 mL), followed by extraction.

[c] The values for MDL and MQL are given by the following equations. MDL = C + 3SD and MQL = C + 10SD. Where C (ng L^{-1}) is the mean and SD (ng L^{-1}) is the standard deviation of the seven blank measurements. Because no blank responses were observed for BDCAA, DBCAA, TBAA, the MDL was calculated from the IDL and the MQL was calculated from the mass of the analyte producing a peak with a signal-to-noise ratio of 10.
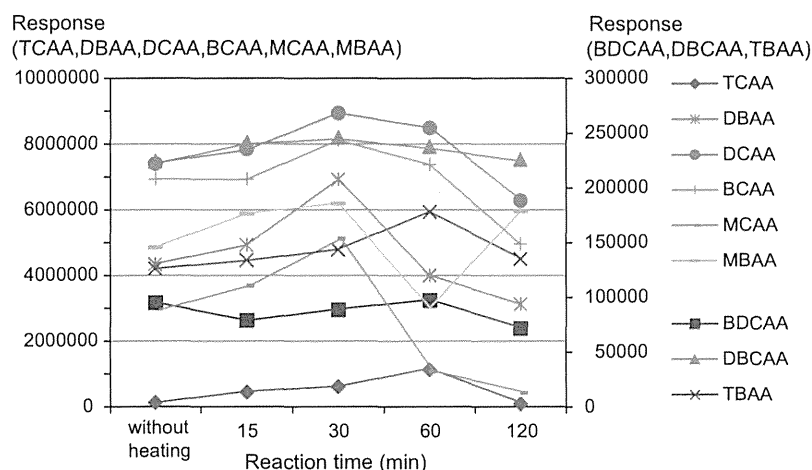
Fig. 2. Time course of the derivatization reaction of HAAs.

three. To estimate the method detection limit (MDL, ng L^{-1}) and MQL (ng L^{-1}), the following equations were used (American Public Health Association, 2012):

$$MDL\,(ng\,L^{-1}) = C + 3SD, \tag{1}$$

$$MQL\,(ng\,L^{-1}) = C + 10SD. \tag{2}$$

where C (ng L^{-1}) is the mean of the blank measurements, and SD (ng L^{-1}) is the standard deviation of the seven blank measurements. Because no blank responses were observed for BDCAA, DBCAA, TBAA, the MDL was calculated from the IDL (signal-to-noise ratio of three) and the MQL was calculated from the mass of the analyte producing a peak with a signal-to-noise ratio of 10. The following equation was used:

$$MDL\,(ng\,L^{-1}) = IDL\,(ng)/2\,(injection\,volume, \mu L)$$
$$\times 500\,(final\,sample\,volume, \mu L)/0.08\,(Initial\,sample\,volume, L). \tag{3}$$

In some earlier studies (Jia et al., 2003), the PFBBr derivatization step for HAAs required 2 h. To examine the time course of HAAs derivatization, a HAAs solution (1 μg in 200 μL) was derivatized without heating or for 15 min, 30 min, 1 h or 2 h on a reaction heater.

2.6. Statistical analysis

Statistical analysis (Student's t-test) was performed using SPSS (version 16.0 for Windows 2007, IBM Corporation, Armonk, NY), with $p < 0.05$ indicating statistical significance.

Concentrations lower than the MDL were given a value of half the MDL for calculation. Concentrations higher than MDL and lower than the MQL were assigned a value halfway between MDL and MQL for the calculation.

3. Results and discussion

3.1. Derivatization

Analytes except for TCAA were reacted with PFBBr without heating while heating was needed for derivatization of TCAA (Fig. 2). Rapid reaction of PFBBr with carboxylic acid at room temperature was reported previously (Michler et al., 1986). The results at 30 min showed the highest responses for most of the target compounds (DCAA, DBCAA, BCAA, DBAA, MBAA, MCAA) and the

second highest responses for TCAA and TBAA (Fig. 2). In addition, the yields of the PFB-ester derivatives decreased with increasing reaction time. This is because the unstable derivatives quickly degrade by decarboxylation to PFB-ethane in time dependent reactions (Jia et al., 2003). Therefore, a reaction time of 30 min was used in subsequent derivatization reactions.

In GC–NCI–MS, the fragment ion [M–CH$_2$C$_6$F$_5$]$^-$ for the MCAA, DCAA, TCAA, MBAA, DBAA, BCAA PFB-esters was observed (Fig. 3a–f). For BDCAA, DBCAA, and TBAA an abundant Br$^-$ ion was observed at m/z 79/81 (Fig. 3g–i), and the IDL ranged from 0.03–0.001 ng. Chromatograms from HAAs analyses are shown in Fig. 3j.

3.2. Method validation

The final procedural blank levels ranged from 6 ng L^{-1} for BCAA to 26 ng L^{-1} for DBAA (Table 2). The MDL and MQL are summarized in Table 2.

The mean recoveries for the spiked samples (n = 5, mean ± SD%) ranged from 89 ± 14% for DBCAA to 99 ± 12% for TCAA (Table 2).

Table 3 compares analytical methods for HAAs in tap water. In the present study, the MDLs of the HAA$_5$ ranged from 0.02 to 0.05 μg L^{-1}, which are comparable to USEPA Method 552.3 using ECD (Domino et al., 2004)

3.3. Application of the developed method to tap water samples

HAAs levels in tap water samples from 15 cities in Japan were quantified by this method. The concentrations of the HAAs were in the range 0.54–7.83 μg L^{-1} (Table 4). The concentrations of HAAs in all of the drinking water samples met the drinking water quality standards (Table 4).

DCAA, TCAA and BCAA were the major HAAs found in all of the drinking water samples, and accounted for 29%, 20% and 19% of the total HAAs, respectively. This is consistent with data reported by Chang (Chang et al., 2010). This composition indicated that bromide was present in water and forms bromine-containing HAAs. By contrast, BDCAA, DBCAA and TBAA were hardly detected in the water samples. Because MDLs for BDCAA, DBCAA and TBAA were relatively high compared with other HAAs, absence of them was not necessarily concluded. A study in Japan showed that levels of BDCAA and DBCAA were comparable to that of BCAA (Kawamoto and Makihata, 2004). Therefore, further investigation with large
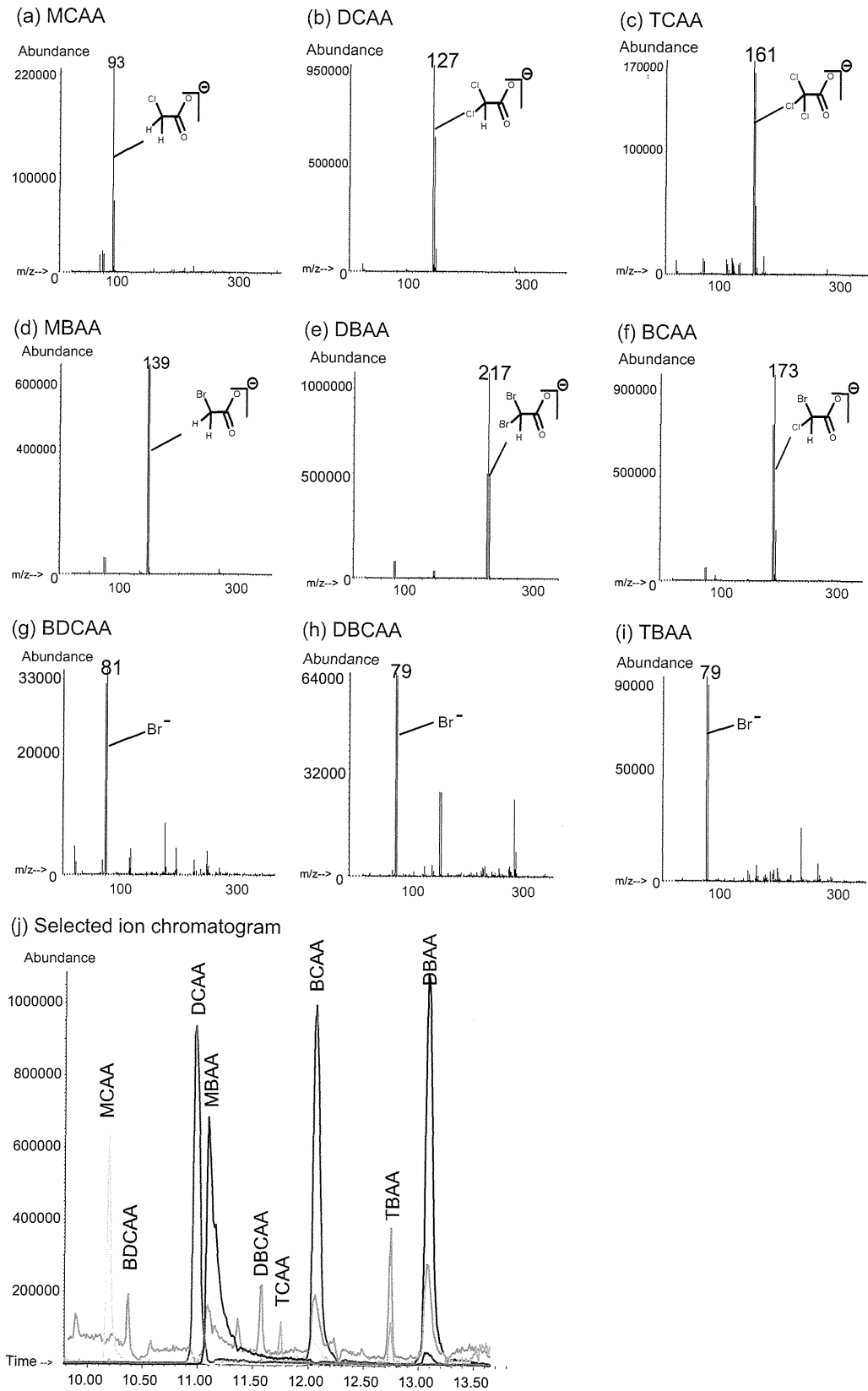
Fig. 3. Mass spectra of HAAs in NCI modes (a–i) and their selected ion chromatogram (j).

Table 3
Comparison of analytical methods for HAAs in tap water samples.

Method	Method detection limits ($\mu g\,L^{-1}$)					Extraction solvent	Derivatization reagent	Instrumental method
	MCAA	DCAA	TCAA	MBAA	DBAA			
This study	0.04	0.04	0.04	0.02	0.05	MTBE	PFBBr	GC–NCI-MS
US EPA method 552.3	0.17	0.02	0.019	0.027	0.012	MTBE	Acidic methanol	GC–ECD [a]

[a] ECD: electron capture detector.

Table 4
HAAs levels in tap water samples collected in 15 cities in Kansai region in Japan.

Area	City	Point	n	Concentration ($\mu g\,L^{-1}$) (mean (range))										
				TCAA	BDCAA	DBCAA	TBAA	DBAA	DCAA	BCAA	MCAA	MBAA	HAA5[c]	HAA9
Shiga	Otsu	1	3	0.57 (0.31–0.71)	ND[a]	ND	Trace[b] (ND–0.26)	0.27 (0.23–0.29)	0.96 (0.63–1.17)	0.57 (0.43–0.65)	Trace (ND–Trace)	0.27 (0.24–0.29)	2.12 (1.44–2.49)	2.91 (2.01–3.48)
Kyoto	Uji	2	3	0.39 (0.21–0.54)	ND	ND	ND	0.20 (0.12–0.35)	0.37 (0.24–0.45)	0.24 (0.22–0.28)	ND	0.21 (0.18–0.23)	1.19 (1.03–1.28)	1.57 (1.45–1.64)
	Joyo	3	3	ND (ND–Trace)	ND	ND	ND	0.11 (ND–0.16)	Trace (ND–0.19)	0.06 (ND–0.15)	ND	0.11 (0.08–0.14)	0.34 (0.15–0.57)	0.54 (0.31–0.86)
	Nagaokakyo	4	1	0.78	ND	ND	0.38	0.38	1.21	0.56	Trace	0.45	2.90	3.93
	Muko	5	1	2.48	0.37	ND	0.29	0.45	2.30	1.21	0.13	0.54	5.91	7.83
	Otokuni	6	1	1.84	ND	ND	0.26	0.65	2.13	1.04	0.12	0.32	5.06	6.45
	Yawata	7	2	2.02 (1.97–2.08)	ND	ND	ND	0.26 (0.23–0.29)	2.48 (2.43–2.53)	0.88 (0.86–0.90)	Trace (Trace–0.10)	0.57 (0.53–0.62)	5.41 (5.24–5.58)	6.44 (6.25–6.63)
Osaka	Ibaraki	8	3	0.59 (0.51–0.64)	ND	ND	Trace (ND–0.33)	0.99 (0.86–1.06)	1.36 (0.98–1.61)	1.23 (0.95–1.38)	0.14 (Trace–0.18)	0.40 (0.36–0.45)	3.48 (2.91–3.93)	5.02 (4.29–5.70)
	Takatsuki	9	3	0.52 (0.36–0.71)	ND	ND	Trace (ND–0.28)	0.87 (0.63–1.07)	1.19 (0.88–1.37)	1.11 (0.82–1.30)	Trace (Trace–0.12)	0.30 (0.26–0.37)	2.97 (2.21–3.37)	4.30 (3.17–5.04)
	Settsu	10	1	0.27	0.47	ND	ND	0.54	0.63	0.62	ND	0.17	1.63	2.81
	Mishima	11	1	0.09	ND	ND	0.25	0.42	0.26	0.30	ND	0.14	0.93	1.58
	Moriguchi	12	1	0.14	ND	ND	ND	0.48	0.50	0.53	Trace	0.28	1.48	2.15
	Kadoma	13	2	0.14 (0.12–0.16)	ND	ND	ND	0.21 (ND–0.39)	0.23 (Trace–0.40)	0.22 (0.02–0.42)	ND	0.20 (0.18–0.22)	0.79 (0.44–1.15)	1.16 (0.60–1.71)
	Neyagawa	14	2	0.11 (0.10–0.12)	ND	ND	ND	0.38 (0.30–0.45)	0.50 (0.38–0.62)	0.44 (0.34–0.55)	Trace (ND–Trace)	0.50 (0.41–0.59)	1.53 (1.23–1.83)	2.12 (1.71–2.52)
	Hirakata	15	3	0.12 (Trace–0.14)	ND	ND	ND	0.33 (0.14–0.60)	0.40 (0.19–0.63)	0.41 (0.16–0.72)	ND (ND–Trace)	0.40 (0.35–0.49)	1.28 (0.84–1.92)	1.83 (1.14–2.78)
	Mean (SD)			0.67 (0.79)	Trace (0.13)	ND	Trace (0.12)	0.44 (0.25)	0.97 (0.78)	0.63 (0.38)	Trace (0.04)	0.32 (0.15)	2.47 (1.77)	3.38 (2.20)
	Range			(ND–2.48)	(ND–0.47)		(ND–0.38)	(ND–1.07)	(ND–2.53)	(ND–1.38)	(ND–0.18)	(0.08–0.62)	(0.17–5.91)	(0.54–7.83)
	Fraction (%)			20	3	1	4	13	29	19	2	9	73	100
Standard for HAAs in drinking water	WHO			200	–	–	–	–	50	–	20	–	–	
	USEPA			–[d]	–	–	–	–	–	–	–	–	60	
	Japan			200	–	–	–	–	40	–	20	–	–	

MDL: method detection limit; MQL: method quantification limit.

[a] If concentration levels are under MDL, the levels are described as ND. Concentrations lower than the MDL were given a value of half the MDL for the caluculation.

[b] If concentration levels are above the MDL and below MQL, the levels are described as trace, and were assigned a value halfway between MDL and MQL for the calculation.

[c] HAA5 = MCAA, DCAA, TCAA, MBAA, DBAA.

[d] –: No data.

volume sample is needed to clarify the contribution of BDCAA, DBCAA and TBAA.

The highest mean HAA levels ($5.27 \pm 1.72\ \mu g\,L^{-1}$) were obtained in drinking water samples 1 and 4–9 (Table 1), which were treated by oxidation with sodium hypochlorite. By comparison, the drinking water samples that were treated by oxidation with ozone contained significantly lower levels of HAAs ($1.72 \pm 0.64\ \mu g\,L^{-1}$) ($p < 0.001$, t-test). Chlorine-containing HAAs showed a significant difference between ozonation and chlorination processes (TCAA: $0.16 \pm 0.14\ \mu g\,L^{-1}$ and $1.01 \pm 0.73\ \mu g\,L^{-1}$; DCAA: $0.34 \pm 0.21\ \mu g\,L^{-1}$ and $1.51 \pm 0.61\ \mu g\,L^{-1}$; BCAA: $0.31 \pm 0.22\ \mu g\,L^{-1}$ and $0.95 \pm 0.31\ \mu g\,L^{-1}$; MCAA: $0.029 \pm 0.016\ \mu g\,L^{-1}$ and $0.098 \pm 0.041\ \mu g\,L^{-1}$, respectively; $p < 0.0001$). In contrast, decrease in MBAA and DBAA levels were moderate in ozonation process ($0.26 \pm 0.15\ \mu g\,L^{-1}$ and $0.27 \pm 0.19\ \mu g\,L^{-1}$, respectively) compared with chlorination process ($0.38 \pm 0.12\ \mu g\,L^{-1}$ and $0.60 \pm 0.33\ \mu g\,L^{-1}$, respectively).

Table 5

Comparison of tap water analysis in the present study with reported data.

Location	n	Concentration (μg L^{-1}) (mean (range))							References
		TCAA	DBAA	DCAA	BCAA	MCAA	MBAA	HAA$_5$	
Seoul, Korea	770	10.7 (ND–34.9)	ND[a]	6.3 (ND–21.6)	–[b]	ND	ND	16 (ND–49.5)	Lee et al. (2013)
China	7	20.7 (8.4–31.2)	6.36 (0.8–20.9)	4.36 (0.3–10.9)	16.5 (ND–39.5)	17.5 (ND–55.3)	5.96 (0.4–35.5)	53.6 (29.6–86.8)	Zhang et al., 2009
Netherlands	20	0.40 (<0.1–1.4)	0.99 (<0.1–6.5)	0.63 (<0.2–3.0)	0.69 (<0.1–2.5)	–	0.11 (<0.1–3.0)	2.82 (<0.3–10.4)	Peters et al. (1991)
Kansai, Japan	30	0.67 (<0.04–2.48)	0.44 (<0.05–1.07)	0.97 (<0.04–2.53)	0.63 (<0.01–1.38)	Trace[c] (<0.04–0.18)	0.32 (0.08–0.62)	2.47 (0.17–5.91)	This study

[a] If concentration levels are under MDL, the levels are described as ND.
[b] –: No data.
[c] If concentration levels are above the MDL and below the MQL, the levels are described as trace.

Bromide ion in water reacts with ozone and forms hypobromous acid, which causes brominated organic by-products (Glaze et al., 1993). Followed by ozonation, water was further treated with activated carbon while the levels of MBAA and DBAA remained comparable to those in chlorination process.

Several studies have investigated HAAs levels around the world (Table 5). The mean concentration of the HAA$_5$ in drinking water in Seoul has been reported as 16.0 μg L^{-1} (range of not detected to 49.5 μg L^{-1}) in 770 samples (Lee et al., 2013). A survey of drinking water in China reported a range of 29.6–86.8 μg L^{-1} for the HAA$_5$ (Liu et al., 2011). The mean level of 2.47 μg L^{-1} for the HAA$_5$ in this study appears low compared with these earlier results in Asian countries. Levels and composition of HAA$_5$ in Netherlands seemed a comparable to current study (Peters et al., 1991).

4. Conclusion

In conclusion, a safe, simple and sensitive method for the determination of HAAs in tap water was developed for GC–NCI–MS with pentafluorobenzyl esterification. The method has a low detection limit (8–94 ng L^{-1}) and good recovery rates (99–89%). This method could be used for routine monitoring of HAAs in drinking water without exposure of workers to occupational hazards.

Acknowledgment

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Methylmercury Monitoring Study in Karakuwacho Peninsula Area in Japan

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Abstract Methylmercury (MeHg) is a worldwide concern owing to its adverse health effects. To explore MeHg exposure burdens and the potential contributing factors in different subpopulations in a peninsula area (Karakuwacho) in Japan, a cross-sectional survey was performed. This study included 189 individuals from 102 families. The geometric means of total hair mercury (THg) were 5.74, 3.78 and 2.37 µg/g for adult males, females and children, respectively, of which 56.5 %, 30.9 % and 12.9 % had hair THg exceeding 5 µg/g, respectively. Tuna and mackerel were the common fish species that were positively correlated with hair THg levels in different subpopulations (standardized coefficient ranged from 0.20 to 0.58, $p < 0.05$). Frequent consumption of these fish species and a large amount of fish intake are likely major contributors of MeHg exposure in this area. Local-scale risk evaluation and risk communication should be highlighted in future studies.

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Mercury is a recognized toxic pollutant of public health concern. Its global distribution is caused by natural processes and anthropogenic activities. Inorganic mercury can be transformed into organic forms (mainly MeHg), which can be bioaccumulated and biomagnified in aquatic food chains (Matthews 1983; Mergler et al. 2007). Various studies have determined that fish consumption is the main source of human exposure to MeHg (Hightower and Moore 2003; Knobeloch et al. 2005). MeHg exposure can lead to various adverse health effects, especially neurological symptoms (Guallar et al. 2002; Harada 1995; Mahaffey 1998; Mozaffarian and Rimm 2006). These adverse health effects may occur at low levels that were previously thought to be safe (Karagas et al. 2012; Maruyama et al. 2012).

In Japan, people habitually consume more fish products than other countries. Historically, several catastrophes (i.e., Minamata disease and Niigata Minamata disease) have been caused by MeHg-contaminated fish consumption (Harada 1995). Several monitoring studies have indicated higher exposure levels in Japan compared with reference values or other populations (Yasuda et al. 2005; Yasutake et al. 2004). Therefore, identifying the high-risk areas and the major contributors is essential for developing effective reduction strategies. In a previous monitoring study (unpublished data), we identified that peninsula or island areas had higher mercury exposure levels. To further investigate the exposure pattern and the potential contributing factors in different subpopulations in such areas, we performed a family-based study of MeHg exposure in Karakuwacho, a peninsula area in the northeast of Miyagi prefecture in Japan.

Materials and Methods

This study was performed in Karakuwacho and included 189 individuals from 102 families. There were ten families composed of father, mother and children; 28 families composed of mother and children; and the other families we collected one individual or two non-parent–child paired individuals. Generally, there were 104 adults (81 females and 23 males) and 85 children (Table 1). For each individual, hair samples (0.1–1.0 g) were cut from the base of the scalp, behind the ear, and the samples were washed with neutral detergents, rinsed twice with acetone, and then dried at room temperature and stored in a desiccator in Kyoto University Human Specimen Bank (Koizumi et al. 2009) until they were analyzed for total mercury (THg). At the time of hair sampling, body weight was measured by a investigator using a standard scale. A questionnaire was administered to the participants to collect information regarding their age, gender, total fish consumption frequency, and commonly consumed fish species. All participants were fully informed about the purposes of the study and provided written consent. The study was approved by the Institutional Review Board and Ethics Committee of the Kyoto University School of Medicine, Japan.

For hair THg analysis, the preconditioned dry hair sample was cut into small pieces (<2 mm) with scissors. Aliquots of samples (15–20 mg) were dissolved in 0.5 mL 2 N NaOH while being heated at 60°C for 1 h. Ten or twenty microliter of the solution was used to analyze the THg levels by the oxygen combustion–gold amalgamation method and an MD-1 atomic absorption detector (Nippon Instruments, Co., Ltd., Osaka, Japan) (Yasutake et al.

2003). This technique of quantification is based on a pyrolysis process of the sample using a combustion tube heated at 700°C under an oxygen atmosphere. Vaporized mercury was transferred to gold-absorber at a carrier gas flow rate of 0.5 L/min. Gaseous compounds other than mercury was eliminated from the system. Gold-absorber was then heated at 700°C for 2 min to vaporize and transfer concentrated mercury to detector. The concentration of mercury was determined by measuring the absorbance of gas at 253.7 nm emitted from mercury-vapor lamp. The limit of detection was 0.1 µg/g-hair (signal to noise ratio: 3) and the limit of quantitation was 0.3 µg/g-hair. All the individuals had hair THg levels above the limit of quantitation. The external standard was 2.5 nM mercuric chloride (0.5 µgHg/mL) in 0.5 M L-cysteine/2 % bovine serum albumin solution. To ensure precision of instrument, standard solution was analyzed in every ten analyses. The analysis was qualitatively confirmed by analyzing a certified reference material of human hair, NIES CRM No. 13 (hair reference material for MeHg, THg and other trace elements, National Institute for Environmental Studies, Japan) with a certified THg value of 4.42 ± 0.2 µg/g (http://www.nies.go.jp/labo/crm/hair.html). The THg level from our method above was 4.55 ± 0.05 µg/g. No detectable contamination was observed from procedural blank samples in every 20 samples. The hair THg levels were compared with various limit levels (1.0, 2.2, 2.7 and 5.0 µg/g) proposed by Japan or other international authorities (US EPA.2000; WHO/JEFCA 2004; The Food Safety Commission, Japan, 2005).

Statistical analysis was performed using STATiSTiCA64 (Supplied by Statsoft, OK, USA). Normally distributed

Table 1 Hair THg levels (µg/g) in 189 individuals in Karakuwacho

Variables	Ten father–mother–children paired families			28 mother–children paired families		Total		
	Fathers	Mothers	Children	Mothers	Children	Adult female	Adult male	Children
No	10	10	15	28	38	81	23	85
Age (years)								
Mean ± SD	40.7 ± 7.2	39.7 ± 5.9	7.8 ± 4.0	35.0 ± 4.8	5.0 ± 3.2	47.2 ± 14.9	43.3 ± 9.8	5.76 ± 3.3
Range	27–50	31–48	2–14	27–47	1–14	21–82	23–63	1–15
Male/female	–	–	–	–	–	–	–	42/43
Hair THg (µg/g)*								
Min	2.21	1.93	0.64	1.12	0.6	1.08	2.21	0.6
Max	12.24	6.79	10.76	13.78	8.38	15.95	14.98	10.76
GM	6.71	3.75	1.92	3.45	2.57	3.78	5.74	2.37
Correlation analysis	F versus M	M versus C	F versus C	M versus C				
Spearman coefficient	0.079	−0.442	0.267	0.290				
p value	0.828	0.200	0.455	0.134				

F versus M, father versus mother; M versus C, mother versus children; F versus C, father versus children

* Kruskal–Wallis test, $p < 0.001$

variants were described by their means and standard deviations. The minimum, maximum, and geometric mean (GM) were used to describe log-normally distributed variants. Non-parametric Kruskal–Wallis tests were conducted to compare THg differences between different populations. Spearman correlation analysis was conducted to explore the relationship between hair THg and fish consumption frequency. Stepwise multiple linear regression analysis was employed to assess the relationship between log THg levels in hair and covariates, such as age, sex, body weight and commonly consumed fish species. The significance level was set at less than 0.05.

Results and Discussion

Mercury, especially MeHg, is a worldwide concern owing to its adverse health effects. In order to ensure the population health, effective reduction strategies should be developed in the high exposure areas. With the aim to identify the detail exposure pattern and the major contributing factors in such a high exposure area, a cross sectional survey was performed in Karakuwacho, a peninsula area in Japan.

Generally, wide variation in hair THg levels was observed for different members of the same family. Fathers had the highest hair mercury level, follow by mothers, and then children (Kruskal–Wallis test, $p = 0.001$). In the same household, there were no correlations in hair mercury levels between family members (correlation analysis, all $p > 0.05$) (Table 1). Considering the potentially different fish consumption patterns, we divided the participants into three subgroups (adult males, adult females and children) for further analysis. Adult males had higher hair THg levels than adult females and children (GMs of THg were 5.74, 3.78 and 2.37 µg/g, respectively, Kruskal–Wallis test, $p < 0.001$) (Table 1). The hair THg levels in this area were significantly higher than the national average levels in Japan (GM values of 2.42 and 1.37 µg/g for males and females, respectively) (Yasutake et al. 2005) and the United States (GM values of 0.12 and 0.20 µg/g for children and women, respectively) (McDowell et al. 2004). There were 30.9 %, 56.5 % and 12.9 % of adult females, adult males and children with hair THg levels that exceeded the least strict limit (5.0 µg/g), respectively (Fig. 1). When considering the at-risk population (women of childbearing age, n = 50), 76 % of them had hair THg exceeding the corresponding limit in Japan (2.7 µg/g), and 26.0 % had hair THg levels exceeding 5.0 µg/g. These levels are significantly higher than levels in other countries (Mahaffey et al. 2009; Kim and Lee 2010). Because MeHg accounts for more than 80 % of THg in hair, THg in hair is thought to be a reliable indicator of MeHg exposure (Cernichiari

et al. 1995). The general population in this area commonly faces high risk of MeHg exposure.

To elucidate the potential contributing risk factors, fish consumption frequency and commonly consumed fish species were investigated. We found that hair THg levels significantly increased with the frequency of fish consumption (Fig. 2). This may explain the hair THg distribution difference between different subpopulations. Adults tended to consume fish more frequently than children; thus, higher hair THg levels were observed (Fig. 2). In present study, we found 25 species of fish were commonly consumed in the survey area (Fig. 3). According to monitoring data released by the government (Online document. MHLW.2010), a majority of these fish species had THg concentrations with less concern (<0.1 ppm), but increasing concern was raised about commonly consumed high-end predatory fish including marlin, tuna, and alfonsino in which the THg concentration was significantly higher than the permitted limit in Japan (>0.4 ppm) (Nakagawa et al. 1997) (Fig. 3).

Multivariate analyses revealed that tuna or canned tuna were the common fish species positively correlated with hair THg levels in different subpopulations (Table 2). Tuna, which is a carnivorous fish with high mercury accumulation, is often consumed in Japan. Yasutake et al. (2004) determined that in the Miyagi area, the average fish consumption was 96 g/person/day, and 69 % of participants in their survey frequently consumed tuna. In the present study, we similarly found that 52.9 % of individuals commonly consumed tuna, and 20.3 % of individuals consumed tuna more than once per week. High tuna consumption tended to be the major contributor to high MeHg exposure in this area.

It is important to note that even fish species contaminated with low levels of mercury, if consumed frequently and eaten in large amounts, may increase the cumulative risk. In the present study, we observed a positive correlation between mackerel consumption and hair THg levels among adult population, even though mackerel has relatively low mercury levels (mean THg level of 0.11 µg/g) (Table 2; Fig. 3). In Japan, the government released a fish advisory in 2003 and further revised it in 2005 (Ser and Watanabe 2012). The advisory mainly focused on ocean fish species. There was no restriction for species with low mercury content. However, in developing a risk reduction strategy, we should consider the contamination level as well as fish consumption amount at the same time.

Although consumption of fish represents a major source of dietary MeHg exposure for the inhabitants of the Karakuwacho peninsula region, this food source also provides high-quality protein and is also rich in unsaturated fatty acids. Consequently, reducing fish consumption as a mean of reducing exposure to MeHg must weigh both the risks and benefits associated with consuming fish as a
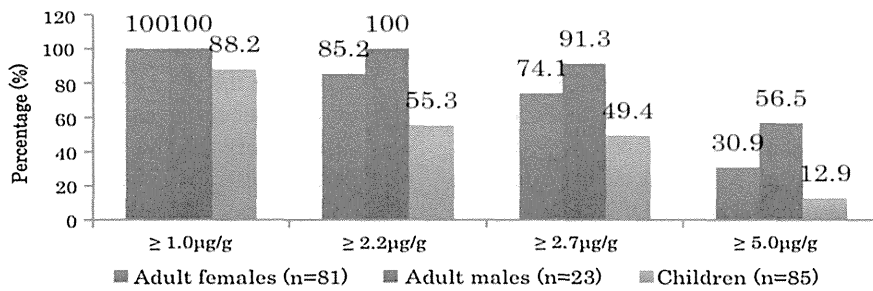
Fig. 1 The percentage of hair THg levels above the limits in different subpopulations of Karakuwacho. Limits: 1.0, 2.2, 2.7 and 5.0 µg/g for hair THg levels correspond to the US EPA, JECFA, and Japanese Food Safety Commission proposed intake limits for pregnant women and the general population (0.7, 1.6, 2.0 and 3.4 µg/kg bw/week), respectively

Fig. 2 Correlation between hair THg levels and fish consumption frequency. Hair THg levels significantly increased with fish consumption frequency. *Boxes* depict 25th, 50th and 75th percentiles, and whiskers depict minimum and maximum values, excluding outliers. *Circles* depict the outliers. The *number* below the *boxes* indicates the proportion of each fish consumption frequency in the corresponding subpopulation
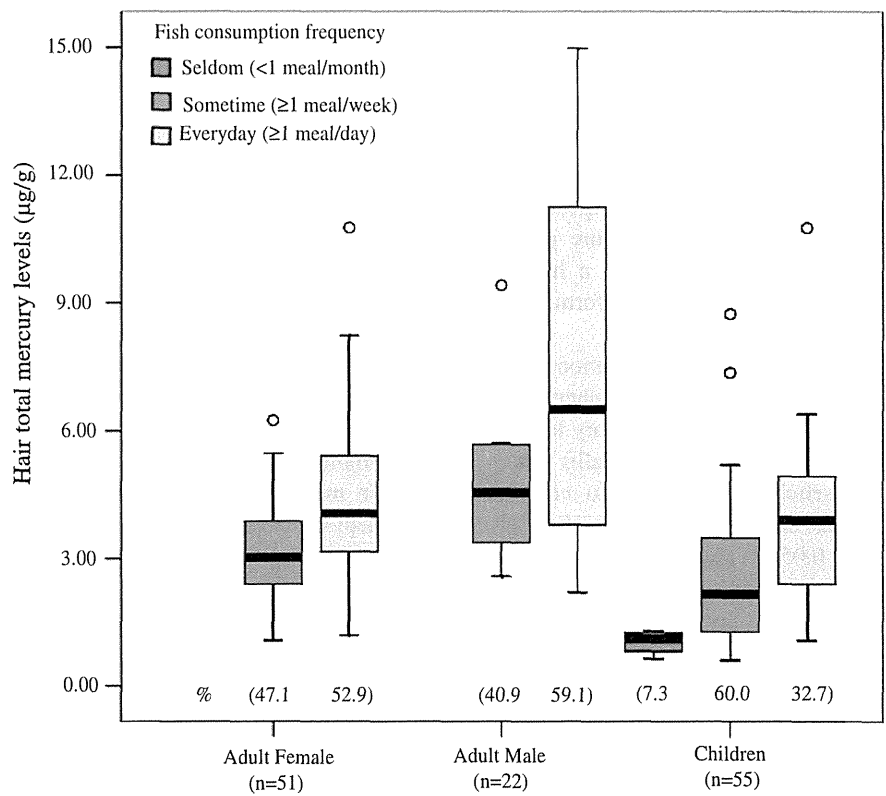
Fig. 3 Fish species commonly consumed by the survey population in Karakuwacho and the means THg concentrations of these fish species, which are cited from the newly released monitoring summary data in Japan (MHLW.2010)
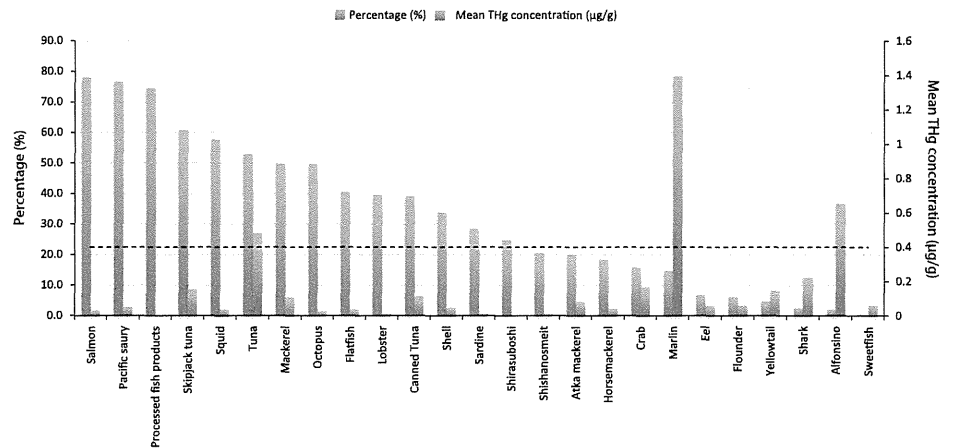
Table 2 Stepwise multiple linear regression analysis between log hair mercury levels and other covariants

Model	Unstandardized coefficients		Standardized coefficients	t	p	Model summary	
	B	SE	B			Adjusted R^2	p
Adult female (n = 81)							
Constant	0.60	0.06	–	10.16	<0.001	0.254	<0.001
Atka mackerel	−0.13	0.06	−0.21	−2.09	0.041		
Tuna	0.15	0.05	0.31	2.94	0.004		
Octopus	0.17	0.05	0.35	3.18	0.002		
Pacific saury	−0.19	0.07	−0.31	−2.66	0.010		
Mackerel	0.12	0.05	0.24	2.24	0.028		
Squid	−0.12	0.06	−0.23	−2.02	0.047		
Children (n = 85)*							
Constant	0.26	0.05	–	5.70	<0.001	0.101	0.002
Tuna	0.21	0.07	0.34	3.16	0.002		
Adult male (n = 23)							
Constant	0.50	0.09	–	5.28	<0.001	0.337	0.008
Canned tuna	0.30	0.09	0.58	3.16	0.005		
Mackerel	0.23	0.10	0.43	2.35	0.030		

Dependent variable: log hair mercury levels. Independent variables: age, body weight, various commonly consumed fish species

SE standard error

* In this regression model, age, sex, body weight and various commonly consumed fish species were included as independent variables

dietary staple. In this study, we determined that pacific saury was negatively correlated with hair THg levels in the female adults (Table 2). According to mercury exposure monitoring data, Pacific saury had a low THg level of 0.052 ppm. Therefore, according to fish consumption patterns and mercury monitoring levels, populations can be guided to choose fish species having low levels of contamination (i.e., salmon and Pacific saury) and avoid species that may contain high mercury levels (i.e., marlin, alfonsino and tuna). Meanwhile, the total amount of fish consumption should always be considered.

In conclusion, the level of MeHg exposure is high in Karakuwacho peninsula region. Consumption of fish with high mercury contamination (e.g., tuna or canned tuna) and high consumption of fish with low mercury contamination (e.g., mackerel) are likely the major contributors. Therefore, both qualitative and quantitative aspects of fish consumption should be addressed to achieve effective reduction in MeHg exposure.

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